Note

Measures of *Bacillus thuringiensis* persistence in the corn whorl

Bacillus thuringiensis Berliner is not toxic to humans and a treated field can be entered immediately. Therefore, viable *B. thuringiensis* spores remaining on the plant, live larvae on the plant, and insecticidal activity of the crystal toxin can be measured over time. When *B. thuringiensis* is applied to corn as a spray or granular formulation, ultraviolet light and rainfall destroy much of the insecticidal activity of the bacterium (Behle et al., 1997). Longevity of *B. thuringiensis* has been measured when applied with over the row equipment (Lynch et al., 1980), but not when placed directly into the corn whorl. The objectives of these studies were to determine the longevity of *B. thuringiensis* spores applied directly into the corn whorl, determine the reliability of the number of live larvae in the whorl to predict centimeters of tunnelling, and determine the insecticidal activity of the *B. thuringiensis* crystal toxin within the whorl of the corn plant.

Pioneer 3541 hybrid corn, susceptible to leaf feeding by *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) larvae, was planted in rows at 55,800 seeds/ha, 0.75 m apart. A randomized complete block with a split-plot arrangement of treatments and six replications was used. Whole-plots (two 40 m long rows) were treated with Dipel ES (emulsifiable concentrate) at 0, 6.6×10^9, and 8.7×10^9 IU/ha (Abbott Laboratories, North Chicago, IL). Split-plots were the day (0, 1, 2, 4, 8, and 12) after *B. thuringiensis* treatment that plants were infested with *O. nubilalis* neonates. Day 0 corresponded to the day *B. thuringiensis* was applied. Plants on day 0 were at the V7 growth stage (Ritchie et al., 1997).

Bacillus thuringiensis was applied to all plants on 7 June, 1988 with a hand held compressed air sprayer to the center of the whorl of each plant. Approximately 50 neonates were applied to the center of the whorl of each plant within a subplot on the appropriate infestation date with a hand held applicator (Davis and Oswalt, 1979). At the time of infestation, whorls from 5 plants/subplot were randomly selected to be analyzed for the presence of *B. thuringiensis* spores. From each plant a section of whorl, 7.6 cm in length, was cut beginning at the point where the leaves were tightly furled and extending upward. Whorls were immediately sealed in a labeled plastic bag and frozen until processed. Presence of *B. thuringiensis* spores was quantified on a per-plant

basis (Lynch et al., 1980). Five days following infestation, whorls were removed from 15 plants/subplot, carefully unrolled, and the number of live larvae counted. Forty days after larval infestation, 25 plants/subplot were split longitudinally from tassel to base and the centimeters of tunnelling recorded.

Virulence of *B. thuringiensis* (crystal protein) was determined using neonate *O. nubilalis* larvae with a droplet assay (Hughes et al., 1986). After visual confirmation that a larva had ingested from the droplets, it was selected. Thirty larvae were transferred individually to laboratory diet without aureomycin (Guthrie et al., 1985) and incubated for 7 days at 27 °C, 50% RH, under constant light, after which mortality was recorded.

Data were analyzed with analysis of variance by using the General Linear Models Procedure and Tukey's multiple range test to separate means (SAS Institute, 1995). Data were analyzed as a split-plot design with *B. thuringiensis* application as the whole-plot and the number of days after *B. thuringiensis* application that neonates were applied to plants as the split-plot. Regression analyses were also performed to determine relationships between variables (SAS Institute, 1995).

There were significant reductions in the number of live larvae ($F = 49.82$; df $= 2$, 15; $P < 0.0001$) and centimeters of tunnelling ($F = 13.66$; df $= 2$, 15; $P = 0.0004$), as well as a significant increase in the number of *B. thuringiensis* spores recovered ($F = 12.48$; df $= 2$, 15; $P = 0.0006$) and larval mortality ($F = 38.73$; df $= 2$, 15; $P < 0.0001$) in *B. thuringiensis*-treated plots and control plots. There were 63 and 62% reductions in the mean number of live larvae and centimeters of tunnelling between the control and the mean of the *B. thuringiensis* applications, respectively. There were significant differences in the number of live larvae ($F = 3.29$; df $= 5$, 75; $P < 0.0001$), centimeters of tunnelling ($F = 2.44$; df $= 5$, 75; $P = 0.04$), number of spores recovered ($F = 7.33$; df $= 5$, 75; $P < 0.0001$), and larval mortality ($F = 28.68$; df $= 5$, 75; $P < 0.0001$) between days of infestation (Table 1). The mean number of live larvae in the whorl of 5 plants was a reliable indicator of centimeters of tunnelling at 40 days ($y = 0.54x + 5.02$; $R^2 = 0.75$; $P < 0.0001$). The mean number of *B. thuringiensis* colony forming units recorded was a reliable indicator of the percent larval

Table 1
Mean (±SEM) larval bioassay mortality and number of *B. thuringiensis* spores recorded

Day	Mortality[a]		Spore count[a]	
	Low[b]	High[c]	Low[b]	High[c]
0	0.38 ± 0.01a	0.51 ± 0.01a	$3.6 \times 10^5 \pm 4.5 \times 10^4$a	$5.7 \times 10^5 \pm 6.1 \times 10^4$a
1	0.36 ± 0.03a	0.42 ± 0.03a	$1.3 \times 10^5 \pm 1.4 \times 10^4$ab	$1.5 \times 10^5 \pm 1.1 \times 10^4$b
2	0.03 ± 0.002b	0.14 ± 0.007b	$1.1 \times 10^4 \pm 1.5 \times 10^3$b	$3.7 \times 10^4 \pm 4.0 \times 10^3$b
4	0.15 ± 0.01b	0.16 ± 0.01b	$9.4 \times 10^4 \pm 2.9 \times 10^3$ab	$1.1 \times 10^4 \pm 6.5 \times 10^3$b
8	0.03 ± 0.003b	0.06 ± 0.005b	$4.5 \times 10^4 \pm 4.0 \times 10^3$ab	$7.4 \times 10^4 \pm 5.1 \times 10^3$b
12	0.03 ± 0.001b	0.05 ± 0.002b	$1.0 \times 10^5 \pm 1.9 \times 10^4$ab	$6.9 \times 10^4 \pm 1.4 \times 10^3$b

[a] Means in the same column with different letters are significantly different ($P \leqslant 0.05$).
[b] *B. thuringiensis* applied at 6.6×10^9 IU/ha.
[c] *B. thuringiensis* applied at 8.7×10^9 IU/ha.

mortality in the bioassay ($y = 9 \times 10^7 x + 0.04$; $R^2 = 0.72$; $P < 0.0001$).

The loss of insecticidal activity over time was indicated by the decrease in larval mortality in the bioassay (Table 1) and an increase in the number of live larvae recorded in the plant whorl. Mean larval mortality was 38 and 51% on day 0 and declined to 3 and 5% on day 12 for the low and high *B. thuringiensis* treatments, respectively. Mean number of live larvae (±SEM) per treatment increased from 8.0 ± 3.0 and 7.5 ± 3.0 on day 0 to 26.0 ± 4.8 and 33.3 ± 1.9 on day 12 for the low and high *B. thuringiensis* treatments, respectively. Larval mortality in the control ranged from 2 to 5% and spore counts ranged from 20 to 864 over the 12 days period. The majority of the reduction in insecticidal activity was observed on day 2 of the bioassay. Larval mortality on day 2 fell to 3 and 14% for the low and high *B. thuringiensis* treatments, respectively. Rainfall totalling 3.1 cm fell 8 June 1988 (1:30–8:30 am), which likely reduced the insecticidal activity of *B. thuringiensis* (Behle et al., 1997; Pinnock et al., 1977). Over the course of the experiment 91% of the maximum sunshine occurred (National Weather Service, Johnston IA). However, the corn whorl has been shown to act as a shield to granular formulated *B. thuringiensis* (McGuire et al., 1994). *B. thuringiensis* sprays are less persistent than granular formulations (Lynch et al., 1980). This may be due to the ability of granules to continue to roll down into the corn whorl as leaves elongate and would limit the amount of sunlight to which granules are exposed. The combination of heavy rain within 18 h of application and intense sunshine dramatically decreased spore persistence. Grinding the corn whorls may have diluted the spore load in the homogenate compared with techniques in which leaf discs of plants treated with *B. thuringiensis* are presented to larvae (Behle et al., 1997). We expected to observe nearly 100% mortality in bioassays of treated plant material performed on the day of treatment, instead we observed a high of 51% mortality. Inactivation of *B. thuringiensis* spores by extracts from plant leaves has been observed (Jaques and Morris, 1981; Krischik et al., 1988), but inactivation due to corn leaf extracts is unknown.

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Leslie C. Lewis[*]
Denny J. Bruck[1]
Robert D. Gunnarson
USDA-ARS
Corn Insects and Crop Genetics Research Unit
Genetics Laboratory
Insectary Building
Iowa State University
Ames, IA 50011
USA
E-mail address: leslewis@iastate.edu
(L.C. Lewis)

[*] Corresponding author. Fax: (515) 294-2265.

[1] Present address: USDA-ARS Horticultural Crops Research Laboratory, 3420 N. W. Orchard Avenue, Corvallis, OR 97330, USA.